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Geomicrobiology of the Ocean Crust: A Role for Chemoautotrophic Fe-Bacteria

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The delicate balance of the major global biogeochemical cycles greatly depends on the transformation of Earth materials at or near its surface. The formation and degradation of rocks, minerals, and organic matter are pivotal for the balance, maintenance, and future of many of these cycles. Microorganisms also play a crucial role, determining the transformation rates, pathways, and end products of these processes. While most of Earth's crust is oceanic rather than terrestrial, few studies have been conducted on ocean crust transformations, particularly those mediated by endolithic (rock-hosted) microbial communities. The biology and geochemistry of deep-sea and sub-seafloor environments are generally more complicated to study than in terrestrial or near-coastal regimes. As a result, fewer, and more targeted, studies usually homing in on specific sites, are most common. We are studying the role of endolithic microorganisms in weathering seafloor crustal materials, including basaltic glass and sulfide minerals, both in the vicinity of seafloor hydrothermal vents and off-axis at unsedimented (young) ridge flanks. We are using molecular phylogenetic surveys and laboratory culture studies to define the size, diversity, physiology, and distribution of microorganisms in the shallow ocean crust. Our data show that an unexpected diversity of microorganisms directly participate in rock weathering at the seafloor, and imply that endolithic microbial communities contribute to rock, mineral, and carbon transformations.

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Weathering reactions in Earth's near-surface environments play pivotal roles in balancing chemical exchange between the lithosphere, hydrosphere, and atmosphere. Microorganisms at and beneath the surface affect the transformation rates, mechanisms, and pathways of exchange for many elements. Over 70% of the Earth's exposed crust is oceanic, and most of this material occurs well below the euphotic upper ocean regime. The oceanic crust is composed largely of basalt (~50 wt % SiO_2; 4–15 wt % each of MgO, FeO, CaO, Al_2O_3), although metal sulfide minerals (*e.g.*, pyrite, FeS_2) are prominent at seafloor hydrothermal ridge axes. After their formation at seafloor spreading centers, ocean crust rocks undergo weathering, either by reaction with seawater while exposed at the ocean floor or from fluid-rock interaction below the seafloor.

While the effects of crustal weathering and fluid-rock interaction are well documented (Alt, 1995), the role of microorganisms in these processes is poorly understood. To date, investigations of sub-seafloor endolithic (rock-hosted) microbial communities have been largely limited to examination of textural features, such as channels and pits, which appear in petrographic thin sections and other microscopic preparations, and are thought to indicate microbial activity (*e.g.*, Fisk *et al.*, 1998). However, the physiological functions of members of sub-seafloor ecosystems have not been elucidated, precluding our ability to place these hypothesized communities into proper global biogeochemical context. Here, we first briefly review the physical and chemical weathering regime of the ocean crust, and then discuss our recent findings regarding the physiological activities and phylogenetic relationships among prokaryotes that actively participate in crustal alteration at the ocean floor.

The uppermost 200–500 m of basaltic ocean crust is characterized by high permeabilities (10^{-12}–10^{-15} m^2) that facilitate the circulation of large quantities of seawater (Fisher,

1998; Fisher and Becker, 2000). Chemical reactions between seawater and seafloor rocks change the compositions of both the oceans and the aging ocean crust (Hart and Staudigel, 1986; Mottl and Wheat, 1994; Alt *et al.*, 1996; Staudigel *et al.*, 1996; Elderfield *et al.*, 1999; de Villiers and Nelson, 1999; Wheat and Mottl, 2000). Basaltic rocks react with oxygenated deep-sea water to form secondary hydrous alteration minerals, including a variety of Fe-oxyhydroxides and micas, and clay minerals such as celadonite ($K(Mg,Fe)(Fe,Al)Si_4O_{10}(OH)_2$) and smectite (*e.g.*, montmorillinite, $(1/2Ca,Na)(Al,Mg,Fe)_4(Si,Al)_8O_{20}(OH)_4{*}nH_2O$)(Honnorez, 1981; Alt, 1995). These minerals replace glass and primary minerals such as olivine ($(Mg, Fe)_2SiO_4$), sulfides (*e.g.*, pyrite), and to lesser extents plagioclase ($NaAlSi_3O_8$) and clinopyroxene ($(Ca,Mg,Fe,Al)_2(Si,Al)_2O_6$), and they fill fractures and void space in the crust. The extent of oxidation associated with low-temperature alteration is extremely variable at different spatial scales. Age dating of celadonite suggests that the products of oxidative alteration persist in crust of significant age (10–40 million years; Peterson *et al.*, 1986; Hart and Staudigel, 1986; Gallahan and Duncan, 1994); these products can be detected and analyzed and the data used to interpret past fluid-rock interactions in ocean crust.

The chemical reactions that occur from fluid-rock interaction in the sub-seafloor not only change the mineralogy of the ocean crust, but also remove many important seawater constituents, such as magnesium and sulfate, while other constituents, such as calcium, are added. These reactions are thus generally responsible for maintaining the chemical composition of the oceans over geological time frames, and they participate in controlling the balance of the greenhouse gases such as CO_2. Because the entire volume of the ocean is circulated through the ocean crust roughly once every million years, we must have a fundamental understanding of the rates, mechanisms, and pathways of ocean crust water-rock reactions so that we may better predict feedbacks such as those between climate change and seawater-crust exchange.

Many of the low-temperature water-rock reactions we have mentioned release energy, yet are kinetically sluggish; consequently, where conditions are otherwise suitable (appropriate temperature, availability of nutrients, etc.), this chemical energy could be used by microorganisms for metabolic growth. Textural observations (Fisk *et al.*, 1998; Torsvik *et al.*, 1998; Furnes and Staudigel, 1999) and highly variable carbon isotope measurements (Furnes *et al.*, 2001) have indeed suggested that microbial activity is present in the ocean crust. These textural criteria—which include recognition and interpretation of "pit-textures," "sponge-textures," and "zoned palagonite"—can be easily and rapidly applied to a large number of samples for qualitative initial screenings and surveys, but they cannot provide definitive evidence of specific microbial activity in the crust. Furthermore, studies of crust-hosted microbial communities have not yet elucidated how they might function physiologically. Hence, the ocean crust is an understudied, yet potentially vast, microbial habitat. Because sample access, contamination, preservation, low biomass, and activity are problematic in the deep sea, many of the usual methods of detecting microbial communities and measuring their activities are not practical. Consequently, the actual fraction of ocean crust that is microbially altered is difficult to estimate. Textural signatures in the alteration products of certain rocks suggest that up to 75% of the uppermost crust is microbially altered (Furnes and Staudigel, 1999), whereas such features in other samples suggest that most alteration is probably abiogenic (Alt and Mata, 2000). Studies of microbial crust alteration have been infrequent, so we cannot conclusively assess the extent and importance of microbial activity within the ocean crust. Hence, the physical, chemical, and energetic regimes of young upper ocean crust must be considered with special care, so that specific predictions may be made and tested for use in focused environmental studies.

To address the inferences made from previous geochemical studies, we have explored microbial weathering reactions that may occur in the upper ocean crust during early-stage (crust $<$10 million years old) oxidative alteration of basaltic glass and sulfide minerals. In July of 2000, *in situ* weathering and colonization experiments were initiated at the Juan de Fuca Ridge axis off the northwestern coast of America (Edwards *et al.*, 2003a). A variety of naturally occurring sulfide minerals were reacted for 2 months at the seafloor at low-temperature ($\sim$3°C), ambient seafloor conditions. Upon collection, the sulfide surfaces were heavily colonized by bacteria and densely encrusted with weathering products that were largely composed of Fe oxides (Fig. 1). The mineralogical and fluorescent hybridization data (FISH; Fig. 1) suggest that these Fe oxide minerals owe their presence to the activity of neutrophilic Fe-oxidizing bacteria within surface pits (Edwards *et al.*, 2003a). Surface pits are ideal colonization sites for aerobic Fe-oxidizing bacteria because, once biofilms have formed and Fe oxide crusts have been produced, the bacteria are partially protected from free advective (initially) and diffusive exchange with well-oxygenated deep-sea water. This allows pit-colonizing, Fe-oxidizing bacteria to more readily compete with very rapid abiotic oxidation kinetics of ferrous iron so that they may harness this oxidation energy for growth.

Both culture-independent (molecular phylogenetic) and culture-dependent studies have been used to further explore the occurrence and diversity of mineral-oxidizing microorganisms. For both types of studies, we examined prokaryotic populations associated with weathering habitats in the deep sea (Fig. 2). These habitats include the surfaces of brecciated sulfide rubble material that derives from collapsed chimney, flange, or other structures common in hydrothermal vent environments. We also examined metalliferous

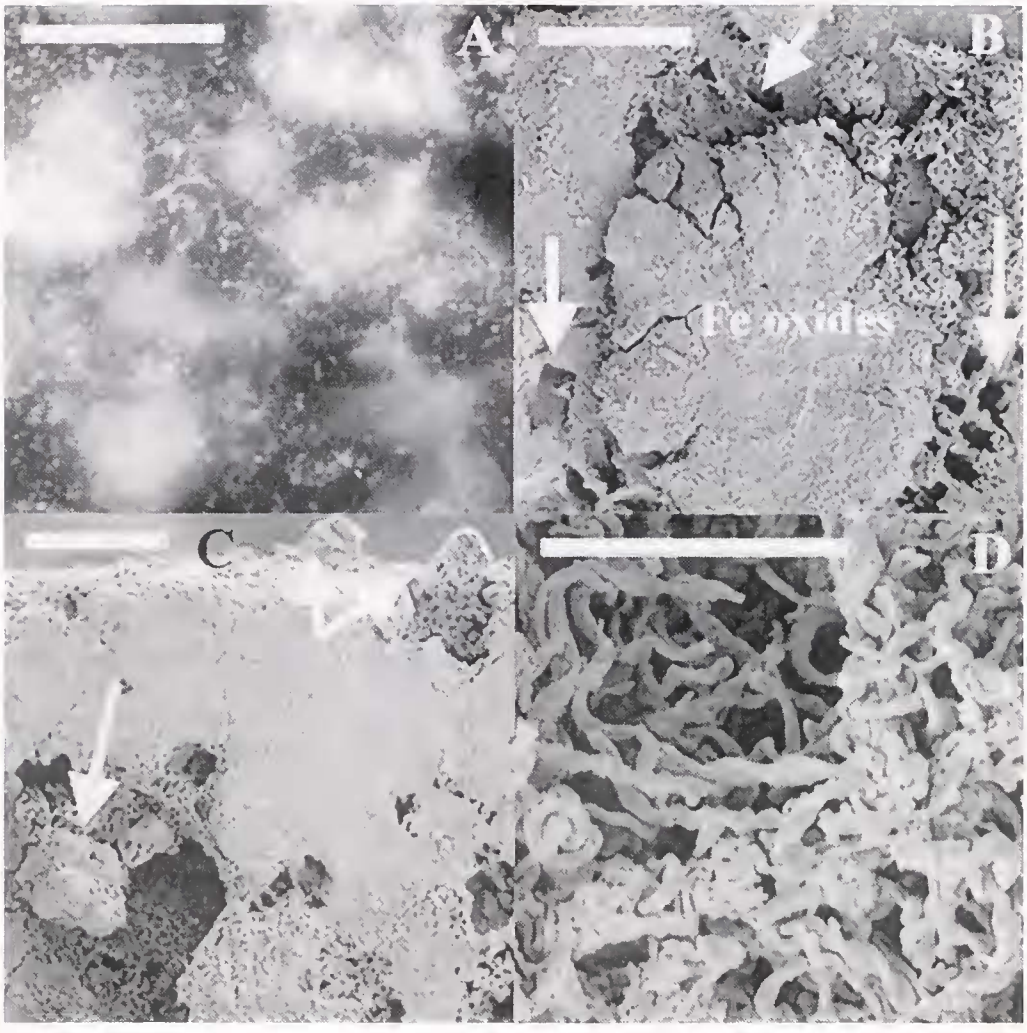

Figure 1. Patterns of cell and oxide accumulation on the surface of a seafloor reacted sulfide mineral. (A) DAPI-stained image of surface that was heavily colonized by Bacteria (determined by fluorescent *in situ* hybridizations; FISH); scale = 50 μm. Cells are bright white dots and patches, predominately colonizing pores and pits on sample, the outlines of which are dark and coincide with the edges of the cell masses. (B,C) scanning electron micrographs of the same surface, showing thick Fe oxide accumulations over top of (B) or within (C) pits and pores on surface; scale = 100 μm. (D) Fe oxides in C at higher magnification; scale = 10 μm. In B, Fe oxides form an effective cap over the pits (arrows). In C, the oxides (arrows) are less well formed and are growing inside pits. We hypothesize that the more unformed Fe oxides in C are the precursors to the massive forms seen in B.

sediments that accumulate in the vicinity of seafloor hydrothermal sites as the result of plume events or the collapse of sulfide-anhydrite structures.

Our culture-independent studies are based on comparative analysis of 16S rDNA sequences from uncultured organisms present in environmental samples, and restriction fragment length polymorphism (RFLP; Hugenholtz *et al.*, 1998). RFLP analyses indicate that all of the weathering habitats examined are characterized by very low microbial diversity. In most cases, the microbial community is dominated by only one to three operational taxonomic units (OTUs; Moyer *et al.*, 1994) or phylogenetically coherent taxonomic groupings as defined by RFLP analysis (D.R. Rogers, C.M. Santelli, and K.J. Edwards, unpubl. data). Comparative analyses of 16S rDNA sequences obtained from these uncultured organisms indicate that sulfur(S)-oxidizing bacteria that are to varying degrees related to the genus *Thiomicrospira* represent one dominant microbial group in these samples (Rogers and Edwards, unpubl. data). These observations are consistent with findings from other studies (both culture-dependent and culture-independent) that have been conducted at seafloor hydrothermal vent sites (*e.g.*, Wirsen *et al.*, 1993), and they support previous inferences that minerals play an important role in supporting the

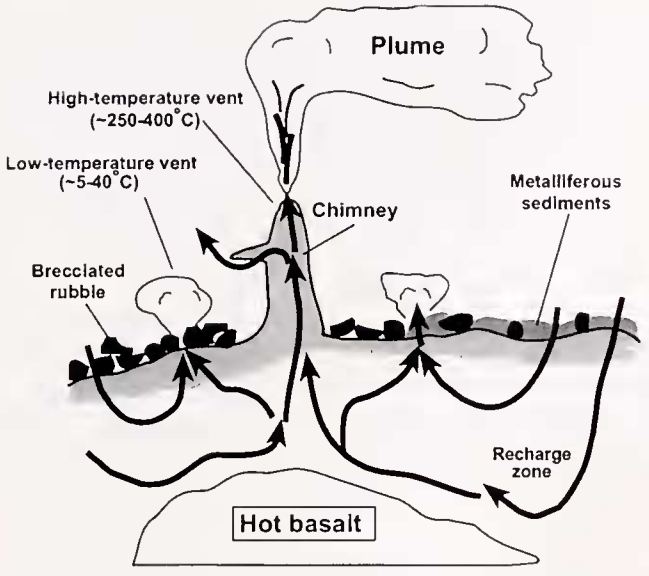

Figure 2. Schematic cross-section of a submarine hydrothermal vent system, emphasizing sulfide weathering habitats. Mineral surfaces exposed to oxygenated water are favorable environments for aerobic lithoautotrophs that can oxidize the minerals to obtain metabolic energy. Such environments include the surfaces of hydrothermal chimneys, brecciated rubble resulting from the collapse of extinct chimneys, and metalliferous sediments formed by particulates settling out of the vent plume. In some instances, mounds of sediments and brecciated rubble are infiltrated by low-temperature hydrothermal fluids formed by mixing of high-temperature fluids and seawater, providing aerobic environments at moderate temperatures (~25–40°C). Figure modified after McCollom (2000) with permission from Elsevier.

growth of S-oxidizing prokaryotes over geological time scales, long after hydrothermal activity dissipates (Eberhard *et al.*, 1995).

In contrast to our FISH studies and microscopic observations (Fig. 1), our culture-independent phylogenetic approaches failed to support our findings that suggest that Fe-oxidizing microorganisms are present in low-temperature weathering deposits. We did not identify any 16S rDNA sequences bearing similarity with gene sequences from any known Fe-oxidizing prokaryotes. This lack of sequence-based support for the presence of Fe-oxidizing microorganisms within environmental samples is not unusual in studies of microbial weathering in the deep sea. For example, Thorseth *et al.* (2001) used both scanning electron microscopy and 16S rDNA sequence analysis on seafloor basalt glass to study microbial weathering at the seafloor. Although their SEM studies reveal Fe oxide particles remarkably similar to those observed in environmental and culture studies of neutrophilic Fe-oxidizing bacteria, their culture-independent, phylogenetic analyses failed to produce any 16S rDNA sequences related to known Fe-oxidizing species.

In contrast to our culture-independent studies, our culture-dependent studies have revealed a wide diversity of novel, autotrophic, Fe-oxidizing bacterial strains that previously had no known Fe-oxidizing or autotrophic relatives represented in pure culture (Edwards *et al.*, 2003b). Our culture techniques are based on the FeS gradient-tube method originally devised by Kucera and Wolfe (1957) and modified by Emerson and Moyer (1997). Using the same samples as were used for our culture-independent studies (above) for inoculum, we first initiated enrichment cultures on an organic carbon-free artificial seawater (ASW; modified after Jannasch *et al.*, 1985; Edwards *et al.*, 2003b) medium, using sulfide minerals such as pyrite as the sole energy source. Following initial enrichment, pure cultures of Fe-oxidizing bacteria were obtained by successive serial dilutions of enrichments to extinction in FeS gradient-tubes (Kucera and Wolfe, 1957). Putative isolates of Fe-oxidizing bacteria were determined to be clonally pure using RFLP analysis (Edwards *et al.*, unpubl. data). Physiological analyses have revealed that all of these isolates are obligate lithotrophs, capable of growth with Fe^{2+}, but not with any alternate electron donors tested so far (sulfide, thiosulfate, Mn^{2+}). Autotrophy has been confirmed by determining ^{14}C fixation in FeS, FeS_2, and basalt (~10 wt. % FeO)-based gradient tubes (Edwards *et al.*, 2003b).

Table 1 shows the phylogenetic affiliations among some of our Fe-oxidizing isolates. Most of our strains fall within the alpha- or gamma-subdivisions of the Proteobacteria and have moderately close relatives within broad groups of known heterotrophic bacteria, the Hypomicrobia and Mari-

Table 1

Phylogenetic affiliations among axenic Fe-oxidizing strains

Strain number	Bacterial division	BLAST database match (% related)	Metabolism inferred from closest relative
FO1	α-Proteobacteria	*Hyphomonas jannaschiana* (81%)	Heterotrophy
FO3	α-Proteobacteria	Uncultured Marine bacterium SCRIPPS_94 (95%)	Heterotrophy
FO4	γ-Proteobacteria	Uncultured Marinobacter sp. PCOB-2 (94%)	Heterotrophy
FO6	γ-Proteobacteria	Uncultured Marinobacter sp. PCOB-2 (95%)	Heterotrophy
FO8	γ-Proteobacteria	Uncultured Marinobacter sp. ME108 (99%)	Heterotrophy
FO10	γ-Proteobacteria	Uncultured DCM-ATT-12 (90%)	Unknown

nobacter, respectively. If these sequences had not been derived from pure cultures of Fe-oxidizing lithoautotrophs, they (and thus their *in situ* physiological activity) would probably have been classified as heterotrophic on the basis of their phylogenetic relationships with known physiological groups.

Our findings indicate that Fe-oxidizing autotrophs may be overlooked in culture-independent studies in the deep sea (if not other habitats as well) due to their close phylogenetic affiliations with physiologically distinct (heterotrophic) species. Our studies and others (*e.g.*, Emerson *et al.*, 1999; Emerson and Moyer, 1997, 2002) clearly indicate that neutrophilic Fe-oxidizing bacteria harbor unexpected diversity, which is just now becoming appreciated. This has important implications for how we study deep-sea microbial communities within the context of their ecological and geochemical functions, and suggests critical shortcomings in the most commonly applied approaches based on 16S rDNA, comparative gene sequence analysis.

The occurrence of Fe-oxidizing capacity among an ever-broadening range of bacteria may have important ecological and evolutionary ramifications. For example, in relatively isolated environments, such as the oligotrophic, low-carbon, bare-rock oceanic crustal habitat, diverse groups of microorganisms may converge on functions that are well suited to the particular environment. Alternatively, the capacity for Fe-oxidation may have been lost by some species after they occupied more carbon-rich habitats. Such occurrences among marine lithoautotrophs may be supported by an alpha-Proteobacterium that possesses genes for both the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), yet the heterotrophic strain has not been shown to fix carbon (Francis *et al.*, 2001). Indeed, although heterotrophic Fe- and Mn-oxidizing bacteria have long been recognized in the environment, the physiological purpose of this oxidation is often unknown (Ghiorse, 1984, and references therein). Rather than serving some explicit biological role, this oxidation in some species may be an evolutionary holdover that no longer has physiological relevance. As one final explanation, Fe-oxidation among groups that we would typically classify as heterotrophic may be an example of a multifunctional metabolism that allows them to adapt to a rapidly changing environment.

Further studies, both laboratory and field-based, are needed to explore the implications of microbial activity within the ocean crust. Studies on Fe-oxidizing bacteria should provide critical information about sub-seafloor communities and biogeochemical processes. Useful laboratory investigations of Fe-oxidizing bacteria would include the following. (1) Studies to determine the mechanism of Fe-oxidation among known strains of Fe-oxidizing bacteria: knowledge of the pathway and key genes and enzymes for Fe-oxidation could be used to develop molecular diagnostics for this activity, and these could be applied in environmental settings. (2) Isotopic studies both to define the carbon fixation pathways and associated stable carbon isotope fractionations and to determine any stable Fe isotope fractionation that occurs during oxidation in pure cultures: these studies are key to developing geochemical diagnostics that can be applied to rocks long after activity diminishes.

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